Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	159	dna adj (pk or activated adj (protein adj kinase$1 or pk))	US-PGPUB; USPAT	OR	OFF	2004/04/30 14:28
L2	49	1 same (substrate$ or peptide$)	US-PGPUB; USPAT	OR	OFF	2004/04/30 14:29
L3	32	1 same (assay$8 or detect$8 or quantit$8)	US-PGPUB; USPAT	OR	OFF	2004/04/30 14:42

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	159	dna adj (pk or activated adj (protein adj kinase$1 or pk))	US-PGPUB; USPAT	OR	OFF	2004/04/30 14:28
L2	49	1 same (substrate$ or peptide$)	US-PGPUB; USPAT	OR	OFF	2004/04/30 14:29

PGPUB-DOCUMENT-NUMBER: 20040039212

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040039212 A1

TITLE: Sphingolipid derivatives and their methods of use

PUBLICATION-DATE: February 26, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 647801

DATE FILED: August 25, 2003

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parent continuation-of 09249211 19990212 US GRANTED

parent-patent 6610835 US

non-provisional-of-provisional 60074536 19980212 US

US-CL-CURRENT: 548/566, 549/491 , 549/74 , 554/36

ABSTRACT:

Derivatives of sphingolipids of the formula: 1
are provided wherein the substituents are as defined in the specification and wherein there is at least one R.sup.2 substituent in the sphingolipid derivative. The compounds are useful in the treatment of of abnormal cell proliferation, including benign and malignant tumors, the promotion of cell differentiation, the induction of apoptosis, the inhibition of protein kinase C, and the treatment of inflammatory conditions, psoriasis, inflammatory bowel disease as well as proliferation of smooth muscle cells in the course of development of plaques in vascular tissue. The invention also includes a method for triggering the release of cytochrome c from mitochondria that includes administering an effective amount of a sphingolipid or its derivative or prodrug to a host in need thereof. Further, the invention provides a method for treating bacterial infections, including those that influence colon cancer and other disorders of the intestine, that includes administering an effective amount of one of the active compounds identified herein.

---------- KWIC --------

Detail Description Paragraph - DETX (586):

[0653] Certain sphingoid bases are known to induce the DNA fragmentation and morphologic features of apoptosis in cancer and leukemia cells. Apoptosis is

the active and gene-directed form of cell death, with well-characterized biochemical and morphologic features. The biochemical features include the generation of lethal and large-sized or endonucleolytic internucleosomal DNA fragmentation. The morphologic features include cell-shrinkage, chromatin condensation and membrane-bound apoptotic bodies. Apoptosis is triggered by the activation of a family of aspartate-specific cysteine proteases designated as caspases. There is a growing list of substrates that are cleaved and degraded by caspases. Among the caspases, the key caspase that is cleaved and activated early in the molecular cascade is caspase-3 (CPP32.beta./Yama). Caspase activity results in the degradation of lamins, poly (ADP-ribose) polymerase (PARP), PKC.delta., Rb protein, DNA-PK, .beta.-actin, fodrin, gel solution, etc., resulting in the morphologic and biochemical features of apoptosis. Recently, it has been demonstrated that caspase-3 cleavage and activity is initiated by the cytosolic accumulation of cytochrome c (cyt c) released from mitochondria, which is triggered by apoptotic stimuli as a pre-apoptotic event. Therefore, promotion of mitochondrial cyt c triggers, while its blockage inhibits, the cleavage and activity of caspase-3 and apoptosis. The gene-product of the anti-apoptotic Bcl-2 gene is localized to the outer mitochondrial membrane, where it blocks the egress of cyt c from mitochondria, thereby preventing the generation of caspase-3 cleavage and activity and resulting apoptosis. Down-regulation of Bcl-2 levels tilts the balance and sensitizes cells to undergo apoptosis.

PGPUB-DOCUMENT-NUMBER: 20040029198

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040029198 A1

TITLE: Method of analyzing ataxia-telangiectasia protein

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 431632

DATE FILED: May 5, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60379841 20020509 US

US-CL-CURRENT: 435/7.23, 435/7.92

ABSTRACT:

The present disclosure concerns methods for recombinantly producing functional ataxia-telangiectasia (ATM) protein, methods for isolating recombinant functional ATM protein, and uses of ATM protein including diagnosing a patient for A-T and/or susceptibility to various conditions including cancer, particularly breast cancer, neurological disorders, and heart disease. Specifically, a method is disclosed for determining the amount of ATM protein in a patient's cells, comparing that amount to a reference, and forming a diagnosis.

RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application No. 60/379,841 entitled METHOD OF ANALYZING ATAXIA-TELANGIECTASIA PROTEIN filed on May 9, 2002. The subject matter of the aforementioned application is hereby incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0006] ATM is a serine/threonine kinase that targets many substrates including p53, RPA, MDM2, NBS1, Chk2, RPA, BRCA1, and other substrates that are postulated but currently unknown. (Gatti et al, (2001) in Metabolic and Molecular Bases of Inherited Disease, 8.sup.th Ed, Scriver et al. Eds, pp 705-732) ATM is a member of a family of large kinases containing a C-terminal end homologous to the phosphatidylinositol 3-kinase domain. These proteins play a role in cell cycle checkpoint or DNA damage repair. Other proteins in this family include Rad 3, Mec1p, Mei-41, Rad 50, Tell and DNA-PK.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040029130 A1

TITLE: Drug screening systems and assays

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 296014

DATE FILED: June 12, 2003

FOREIGN-APPL-PRIORITY-DATA:

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GB	0012179.8	2000GB-0012179.8	May 20, 2000

PCT-DATA:
APPL-NO: PCT/GB01/02180
DATE-FILED: May 18, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6, 435/91.2

ABSTRACT:

A method of stimulating non-homologous end-joining (NHEJ) of DNA the method comprising performing NHEJ of DNA in the presence of inositol hexakisphosphate (IP.sub.6) or other stimulatory inositol phosphate. An assay of a protein kinase wherein the assay comprises inositol hexakisphosphate (IP.sub.6) or other stimulatory inositol phosphate. The invention also provides screening assays for compounds which may modulate NHEJ and which may be therapeutically useful; and screening assays for compounds which may modulate DNA-PK and related protein kinases and which may be therapeutically useful. Methods of modulating NHEJ and protein kinases are also disclosed.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (14):
[0014] Hall-Jackson et al (1999) Oncogene 18, 6707-6713 shows that ATR is a caffeine-sensitive, DNA-activated protein kinase with a substrate specificity distinct from DNA-PK

Summary of Invention Paragraph - BSTX (24):
[0024] "Non-homologous end-joining" is the ligation of DNA termini,

typically intermolecular ligation. It includes the joining of DNA ends which exhibit little or no complementarity to each other (and so, typically, each end does not hybridise to the other) and, in any event, is a term well known in the art as is evidenced by its use in many of the papers and patent applications referred to herein, all of which are incorporated herein by reference. Typically, a NHEJ reaction requires a suitable DNA substrate, and suitable components for the reaction of joining the DNA ends to proceed. Suitable DNA substrates are those that, typically, are linear DNA molecules the length of which need only be large enough to accommodate the factors which participate in NHEJ. Conveniently, each DNA fragment to be joined is, independently, at least 50 bp, preferably at least 70 bp, more preferably at least 100 bp but may be bigger. In relation to the observation of a NHEJ reaction, particularly in a screening assay, one or both of the DNA molecules (or DNA ends) to be joined are detectably labelled such as with radiolabelled phosphorus or with fluorescent labels. Although it is convenient to use two separate DNA molecules to be joined in the NHEJ, two ends of the same molecule can be joined such as the ends of a linearised plasmid. NHEJ typically takes place in a eukaryotic cell, such as a vertebrate cell including mammalian cells (although it can also occur in some circumstances in prokaryotes) but, as is described in detail in Baumann & West (1998) Proc. Natl. Acad. Sci USA 95, 14066-14070, it can also occur in cell-free extracts, such as those obtained from human cells as therein described. Intermolecular ligation in this cell-free system was found to be accurate and to depend on DNA ligase IV, XRCC4 and DNA-dependent protein kinase (DNA-PK; this is a heterotrimer made up of a catalytic subunit DNA-PKcs (encoded by the XRCC7 gene) and two further subunits which are believed to be involved in DNA binding, namely Ku70 and Ku80 subunits (which are encoded by the XRCC6 and XRCC5 genes, respectively). However, it is possible to get a low level of NHEJ with DNA ligase IV and XRCC4 in the absence of DNA-PK, but a greater extent of NHEJ is obtained when DNA ligase IV and XRCC4 are present with Ku70 and Ku80, and still further NHEJ is achieved when the catalytic subunit of DNA-PK is present. Following the inventors present work, it has now been shown that NHEJ is far better in the presence of IP.sub.6, and even better in the presence of IP.sub.7.

Summary of Invention Paragraph - BSTX (25):

[0025] By "stimulating NHEJ" we include the meaning that the rate of NHEJ of DNA is increased by the presence of IP.sub.6 in a NHEJ reaction mixture compared to the rate when IP.sub.6 is not present and the reaction mixture is otherwise the same. It will be appreciated that the stimulation will reach a threshold level and that, typically, stimulation according to the method is achieved when IP.sub.6 or other stimulatory inositol phosphate is included in a NHEJ reaction to which no IP.sub.6 or other stimulatory inositol phosphate has been added previously. Although not being bound by any theory, it is possible that the presence of IP.sub.6 or other stimulatory inositol phosphate is essential for NHEJ and so the presence of IP.sub.6 or other stimulatory inositol phosphate may stimulate a NHEJ reaction from there being no joining to there being some joining of substrate DNA. It is noted that human cell-free extracts can perform NHEJ in the presence of a suitable substrate, as described in Baumann & West (1998) Proc. Natl. Acad. Sci. USA 95, 14066-14070, without the addition of IP.sub.6; however, in this instance it is possible that the cell-free extract already contains a small amount of IP.sub.6 or other stimulatory inositol phosphate. We have now shown that when this cell-free extract is fractionated during partial purification it loses its ability to carry out NHEJ efficiently despite containing the relevant protein components. Addition of IP.sub.6 or IP.sub.5 or IP.sub.4 to the fraction (termed "PC-C" in Example 1) derived from the human cell-free extract has been shown to stimulate NHEJ of DNA. Although not being bound by any theory, it is believed that, by its chemical nature, IP.sub.6 (and other inositol phosphates) flow through the phosphocellulose column used in the preparation of the PC-C fraction separating

it from DNA-PK, XRCC4 and DNA ligase IV (see Example 1).

Summary of Invention Paragraph - BSTX (45):
[0045] The assay contains sufficient components in order to carry out NHEJ of DNA. In particular, typical assays of NHEJ are those which can be performed in vitro such as described in Baumann & West supra. NHEJ in cell-free systems is also described in Labhart (1999) Eur. J. Biochem. 265, 849-861, incorporated herein by reference. Reconstitution of NHEJ may be achieved by using recombinantly expressed protein components (such as expressed using a baculovirus system); typically, such a reconstituted system includes DNA-PK, XRCC4, DNA ligase IV, a suitable DNA substrate and a stimulatory inositol phosphate such as IP.sub.6. The assay may also be carried out in vivo using DNA substrates which, for example, are designed to observe V(D))J joining (see Smith et al (1998) J. Mol. Biol. 281, 815-825 for an example).

Summary of Invention Paragraph - BSTX (60):
[0060] A third aspect of the invention provides the use of IP.sub.6 or other stimulatory inositol phosphate for stimulating non-homologous end-joining of DNA. It is will be appreciated that before the present invention, and despite the extensive study of NHEJ, it was not realised that IP.sub.6 or other stimulatory inositol phosphate could stimulate (or may even be essential for) NHEJ. The invention also provides the use of IP.sub.6 or other stimulatory inositol phosphate in assays for compounds which modulate NHEJ by whatever means, and in methods which modulate NHEJ by whatever means. The assays may involve changes in NHEJ activity, changes in the recognition of substrates by the NHEJ components and/or changes in subcellular localisation of components of the NHEJ reaction such as DNA-PK, XRCC4 or DNA ligase IV.

Summary of Invention Paragraph - BSTX (97):
[0097] Typically the NHEJ of DNA is carried out in a NHEJ reaction mixture which includes DNA-PK, XRCC4, DNA ligase IV and a suitable DNA substrate. Preferably, DNA-PK, XRCC4 and DNA ligase IV are the only polypeptide components of the reaction mixture. Preferably, the DNA-PK, XRCC4 and DNA ligase IV are each substantially pure (eg at least 90% pure) before combination.

Summary of Invention Paragraph - BSTX (103):
[0103] In a preferred embodiment of the invention the components of the NHEJ reaction between which an interaction is measured is any one or more of a DNA-dependent protein kinase (or its components ie DNA-PK.sub.cs, Ku70 and Ku80), XRCC4, DNA ligase IV and a suitable DNA substrate. Other interactions include interaction of any one of XRCC4, DNA ligase IV and the DNA substrate with any one of Mre11, NBS and Rad50 which themselves form a complex (Mre11/NBS/Rad50). The Mre11/NBS/Rad50 complex is believed to net act upstream of DNA-PK in the processing of DNA ends. Further details of the Mre11/NBS/Rad50 complex are found in Labhart (1999) Eur. J. Biochem. 265, 849-861.

Summary of Invention Paragraph - BSTX (107):
[0107] Preferred embodiments of this aspect of the invention measure the interaction (in the presence of IP.sub.6 or other stimulatory inositol phosphate) between DNA-PK and its DNA effector; DNA-PK and a cosubstrate (such as ATP); DNA-PK and XRCC4; DNA-PK and DNA ligase IV; XRCC4 and DNA ligase IV; and DNA ligase IV and its DNA substrate and/or a cosubstrate such as ATP. Further preferred embodiments of this aspect of the invention measure the interaction (in the presence of IP.sub.6 or other stimulatory inositol phosphate) of Ku70/80 with DNA-PK.sub.cs, and the interaction between Ku70 and Ku80 (whether or not in the presence of DNA-PK.sub.cs). The interaction between Ku70/80 and DNA-PK.sub.cs may require DNA.

Summary of Invention Paragraph - BSTX (109):

[0109] The measurement of interactions between certain of these components is described in WO 98/30902 and these measurement techniques are incorporated herein by reference. It should be noted that, as discussed above, there is no realisation in WO 98/30902.of the importance of IP.sub.6 or other stimulatory inositol phosphate in NHEJ of DNA or in the interaction between components required for NHEJ. In this aspect of the invention, it is particularly preferred if the method is used to identify compounds which modulate or mimic the effect of IP.sub.6 or other stimulatory inositol phosphate in stimulating NHEJ by altering the interaction of DNA-PK with another component (such as its effector DNA or DNA ligase IV or XRCC4 or its cognate DNA or a substrate or cosubstrate) or by altering the interactions between the components of DNA-PK (ie DNA-PKcs and Ku70 and/or Ku80).

Summary of Invention Paragraph - BSTX (113):

[0113] DNA-PK is known to phosphorylate XRCC4 which is, therefore, a suitable substrate. Peptide portions of XRCC4 may also be suitable as substrates.

Summary of Invention Paragraph - BSTX (114):

[0114] Each of DNA-PK, ATR, ATM and FRAP can phosphorylate p53 in vitro. This phosphorylation has been mapped to Ser15 in p53 for ATM, ATR and DNA-PK so an N-terminal peptide of p53 may be used as a substrate (Hall-Jackson et al (1999) and references cited therein). Yarosh et al (2000) J. Invert. Dermatol. 114, 1005-1010 shows that FRAP is a DNA-dependent protein kinase which is associated with UV-induced damage. FRAP also phosphorylates PHAS-1 (Brunn et al (1997) Science 277, 99-101).

Summary of Invention Paragraph - BSTX (115):

[0115] Catalytic activity of a protein kinase, such as DNA-PK, ATR, ATM or FRAP, can readily be determined using methods well known in the art, such as by measuring the incorporation of a radiolabelled phosphate group in the substrate following transfer from ATP. Typically, a series of reactions are carried out which assess the effect of the test compound in order to confirm (or deny) that it is a compound which modulates or mimics the effect of IP.sub.6 or other stimulatory inositol phosphate on a protein kinase rather than a compound which has a non-specific effect. Thus, for examples the test compound may be added to the protein kinase reaction either before or after the addition of IP.sub.6 or other stimulatory inositol phosphate to determine whether the order of addition has an effect on the catalytic activity. Alternatively, or additionally, comparisons may be made between a reaction which contains the test compound and IP.sub.6 or other stimulatory inositol phosphate and reactions which do not contain IP.sub.6 or other stimulatory inositol phosphate or do not contain the test compound.

Summary of Invention Paragraph - BSTX (119):

[0119] Typically, a series of measurements are made which assess the effect of the test compound in order to confirm (or deny) that it is a compound which specifically modulates or mimics the effect of IP.sub.6 or other stimulatory inositol phosphate on the protein kinase rather than a compound that has a non-specific effect. Thus, for example, the test compound may be added to a sample containing the protein kinase and a compound with which it interacts (such as its substrate but in the absence of ATP or such as an effector DNA as is the case with DNA-PK or ATR or ATM or FRAP) before or after the addition of IP.sub.6 or other stimulatory inositol phosphate to determine whether the order of addition has an effect on the interaction between components. Alternatively, or additionally, comparisons may be made between the interactions in a sample containing the protein kinase and a component with which it interacts which sample contains the test compound and IP.sub.6 or

other stimulatory inositol phosphate with equivalent samples (in terms of protein kinase and interacting component) which do not contain IP.sub.6 or other stimulatory inositol phosphate or do not contain the test compound.

Detail Description Paragraph - DETX (27):

[0213] The consequences of IP.sub.6 binding by DNA-PK might be structural in nature, possibly due to an allosteric shift upon association with IP.sub.6. Alternatively, binding of IP.sub.6 could simply alter the surface charge distribution of DNAPK Such an alteration of local electrostatic potential has been observed in the binding of IP.sub.6 to phosphoglycerate mutase (Rigden et al, 1999). In this case, ligand binding was mediated by both hydrogen bonding interactions and by the strong positive electrostatic potential of the active site cleft. Occupancy of this highly charged cleft by IP.sub.6 exposes several phosphates to the solvent, which has a pronounced effect on the local electrostatic potential relative to the unbound state. An alteration of this kind would result in a more passive transition from the NHEJ-inactive to the NHEJ-active state of DNA-PK. Of course these two possibilities are not mutually exclusive and both mechanisms might influence the substrate specificity of DNA-PK as well as its potential to participate in extensive protein complexes.

Detail Description Paragraph - DETX (28):

[0214] As far as we are aware, DNA-PK is the only protein known to participate in NHEJ that has been demonstrated to bind IP.sub.6. DNA-PK is a member of the phosphatidylinositol 3-kinase (PI3K)-related kinase family, as are ATM and ATR. All three proteins exhibit a strong sequence homology to the PI 3-kinases, especially in the catalytic core domain that binds and phosphorylates the phosphoinositol headgroup of phosphatidylinositol. However, no phosphorylation of lipid substrates has been observed by DNA-PK.sub.cs, or by other members of this PI3K-related family of kinases (Carpenter and Cantley, 1996; Hunter, 1995; Keith and Schreiber, 1995; Wymann and Pirola, 1998).

PGPUB-DOCUMENT-NUMBER: 20040014701

PGPUB-FILING-TYPE: new

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TITLE: Inhibiting retrotransposon and retroviral integration by targeting the atm pathway

PUBLICATION-DATE: January 22, 2004

INVENTOR-INFORMATION:

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102(E)-DATE:

US-CL-CURRENT: 514/44, 435/5 , 435/6

ABSTRACT:

Ataxia telangiectasia mutated (ATM)-dependent DNA damage signalling mechanisms are involved in retroviral and retrotransposon integration. Screening methods for inhibitors of retroviral and retrotranspons activity comprise inhibiting the ATM-dependent DNA damage signalling pathway, e.g. by disrupting interaction between components of the pathway. Inhibitors are useful as anti-retroviral agents, e.g. in inhibition of HIV.

---------- KWIC ---------

Detail Description Paragraph - DETX (11):

[0117] DNA-PK was purified from HeLa nuclear extract as described previously (Gell et al., 1999). ATM was immunoprecipitated from HeLa nuclear extract using polyclonal antisera raised to the caspase cleavage site region of ATM as described previously (Smith et al., 1999). Kinase assays were performed in 50 mM Hepes, pH 7.5, 50 mM KCl, 4 mM MnCl.sub.2, 6 mM Mg Cl.sub.2, 10% glycerol, 1 mM DTT, 1 mM NaF and 1 mM NaVO4 containing either purified DNA-PK or immunoprecipitated ATM and 1 .mu.g of the substrate GST-p53 (residues 1 to 66). Reactions were pre-incubated at 30.degree. C. for 10 minutes with varying concentrations of wortmannin or LY294002 (for final concentrations see figure). This was followed by the addition of 5 .mu.Ci of .gamma.[.sup.33P]-ATP and ATP to a final concentration of 50 .mu.M. Reactions were then incubated for a further 20 minutes at 30.degree. C. before stopping them with SDS-PAGE sample buffer. Proteins were separated by SDS-PAGE and the gels dried before using

autoradiography to detect phosphorylated substrate.

Detail Description Paragraph - DETX (33):
[0139] In order to determine if ATM represents the additional target responsible for the differential activity of these two compounds in SCID cells, we looked at the effects of both wortmannin (FIG. 6A) and LY294002 (FIG. 6B) on the kinase activity of ATM. In these biochemical assays, purified DNA-PK and ATM proteins were tested in parallel for their ability to phosphorylate a bacterially expressed GST-p53 substrate in the presence of increasing concentrations of wortmannin or LY294002 (see Materials and Methods). Kinase reactions containing either purified DNA-PK or immunoprecipitated ATM and GST-p53 substrate (p53 residues 1 to 66) were performed in the presence of varying concentrations of wortmannin or LY294002 as indicated in FIG. 6.

Detail Description Paragraph - DETX (34):
[0140] FIG. 6A shows that phosphorylation of the p53 substrate by DNA-PK is almost completely inhibited in vitro at concentrations of wortmannin of 0.25 .mu.M. ATM kinase activity is also abolished by wortmannin, as previously described (Moyal et al., 1998; Sakaria et al., 1998; Smith et al., 1999), although this occurs at the higher concentration of 5 .mu.M. Notably, the compound LY294002, while completely inhibiting DNA-PK activity at a concentration of 50 .mu.M as previously described (Izzard et al., 1999), shows virtually no inhibitory capacity against ATM, even at concentrations far greater than those inhibiting DNA-PK activity. These data demonstrate the differential effects of wortmannin and LY294002 on ATM. They also provide a potential explanation for the observed differences between wortmannin and LY294002 in inhibiting residual retroviral integration in SCID cells (see below).

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040014652 A1

TITLE: Tumor activated prodrug compounds and methods of making and using the same

PUBLICATION-DATE: January 22, 2004

INVENTOR-INFORMATION:

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DATE FILED: June 16, 2003

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PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/12, 530/324 , 530/350

ABSTRACT:

The invention is directed to novel prodrug compounds, compositions comprising the prodrug compounds, methods of making the prodrug compounds and methods of using the prodrug compounds. The prodrug compounds comprise a biologically active entity linked to a masking moiety via a linking moiety. The prodrug compounds are selectively activated at or near target cells and display lower toxicity and possibly a longer in vivo or serum half-life than the corresponding naked biologically active entity.

---------- KWIC ---------

Detail Description Paragraph - DETX (111):
[0140] Granzyme B, a single-chain serine protease of about 28.5 kDa, was first demonstrated to play a crucial role in the initiation of apoptosis induced by killer lymphocytes. This killing effect results from the synergistic effect of perforin, a membranolytic protein and the serine protease granzyme B (Blink et al., 1999, Immunol. Cell Biol. 77:206-215; Trapani et al., 1998, J. Biol. Chem. 273:27934-27938). Perforin allows granzyme B to reach the cytoplasm and the nucleus of cells by inducing the formation of

transmembrane pores that constitute a passage for the enzyme. Granzyme B then induces apoptosis by starting pre-existing death pathways through the enzymatic cleavage and activation of pro-caspases, and also by directly cleaving nuclear substrates such as DNA-PK and poly-ADP ribose polymerase (Froelich et al., 1996, Biochem. Biophys. Res. Commun. 227:658-665; Yang et al., 1998, J. Biol. Chem. 273:34278-34283). In the prodrug, the transport peptide potentially plays the role of perforin by allowing granzyme B to enter the cell and to induce apoptosis.

PGPUB-DOCUMENT-NUMBER: 20040005586

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040005586 A1

TITLE: Novel Artemis/DNA-dependent protein kinase complex and methods of use thereof

PUBLICATION-DATE: January 8, 2004

INVENTOR-INFORMATION:

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Schwarz, Klaus	Ulm		DE	

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DATE FILED: February 5, 2003

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non-provisional-of-provisional 60360659 20020228 US

US-CL-CURRENT: 435/6, 435/199 , 435/91.2

ABSTRACT:

In the present invention, it is disclosed that Artemis forms a complex with the 469 kDa DNA-dependent protein kinase (DNA-PK.sub.cs) in vitro and in vivo in the absence of DNA. The purified Artemis protein alone possesses single-strand specific 5' to 3' exonuclease activity. Upon complex formation, DNA-PK.sub.cs phosphorylates Artemis, and Artemis acquires endonucleolytic activity with respect to single-stranded nucleotides, including 5' and 3' overhangs, as well as hairpins. Further, the Artemis:DNA-PKcs complex can open hairpins generated by the RAG complex from a 12/23-substrate pair. Thus, DNA-PK.sub.cs regulates Artemis by both phosphorylation and complex formation to permit enzymatic activities that are critical for the hairpin opening step of V(D)J recombination and for all of the 5' and 3' overhang processing in nonhomologous DNA end joining.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 60/355,452, filed Feb. 6, 2002, and to U.S. Provisional Application Serial No. 60/360,659, filed Feb. 28, 2002.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (10):

[0043] FIG. 5A is an image of a gel in which a 20 bp hairpin (D.sub.FL16.1) with a 1-nucleotide 5' overhang labeled at the 5' end with T4 PNK was used as

the substrate. In reactions with inhibitors, DNA-PK was either mock treated with DMSO (lane 6) or treated with LY294002 at 50 .mu.M (lane 7) and 100 .mu.M (lane 8) first, and then the substrate was added.

Detail Description Paragraph - DETX (98):

[0138] Nuclease assays without RAGs were carried out in a total volume of 10 .mu.L with a buffer composition of 25 mM Tris, pH 8.0, 10-50 mM NaCl or KCl, 10 mM MgCl.sub.2, 1 mM DTT, and 50 ng/.mu.L of BSA unless otherwise specified. To the buffer mixture, Artemis was added to 2.75 pmol, and DNA-PK.sub.cs and Ku were added to 1.25 pmol each. 0.25 mM of ATP (or ADP, ATP-y-S, AMP-PNP) and 0.5 PM of 35 bp DNA were included where DNA-PK.sub.cs was used. Reactions were incubated at 37.degree. C. for 30 minutes. In reactions including DNA-PK.sub.cs inhibitors, reaction mixtures without the substrate were incubated on ice for 15 minutes before the addition of the substrate and the subsequent incubation at 37.degree. C. In FIG. 7, pre-phosphorylation of Artemis-myc-His immunobeads was carried out under DNA-PK kinase assay conditions. After washing the treated immunobeads with buffer F for three times and the nuclease assay buffer for two times, the beads were used for the nuclease reactions. In the hairpin opening of RAG-generated hairpins (FIG. 8), the reactions contained 25 mM K-HEPES, pH 7.4, 50 mM KCl, 10 mM MgCl.sub.2, 1 mM DTT, 0.25 pmol of labeled 12-RSS double-stranded oligonucleotides (KY28/KY29) and an equal amount of unlabeled 23-RSS double-stranded oligonucleotides (KY36/KY37), 1 pmol of RAGs (assuming that the RAG complex consists of two RAG-1 and two RAG-2 subunits), 2 pmol of HMG1, 2.75 pmol of Artemis-myc-His, and 1.25 pmol of DNA-PK.sub.cs (with ATP and 35 bp DNA as described above). For the sequential reactions, substrates were incubated with RAG complex alone first at 37.degree. C. for 60 minutes, extracted with or without phenol/chloroform, then Artemis-myc-His and DNA-PK.sub.cs were added, followed by another 30-minute incubation at 37.degree. C. Reactions with the RAG complex, Artemis, and DNA-PK.sub.cs added simultaneously were incubated for 90 minutes at the same temperature. After incubation, reactions were stopped by adding an equal volume of formamide gel loading buffer and heating at 100.degree. C. for 5 minutes. DNA was resolved on 12% denaturing polyacrylamide gels. The gels were then dried and exposed to a PhosphorImager screen. Data was analyzed by ImageQuant software (v5.0).

Detail Description Paragraph - DETX (115):

[0147] A DNA-PK kinase assay was performed to determine whether Artemis is a phosphorylation substrate of DNA-PK.sub.cs. The results are shown in FIG. 10. DNA-PK.sub.cs was incubated alone (i.e., with no protein substrate; lanes 1 and 2), or with DNA ligase IV/XRCC4 (positive control, lanes 3 and 4) or GST-Artemis (lanes 5 and 6). The low amount of XRCC4 and Artemis phosphorylation in the absence of 35 bp dsDNA is thought to be due to a low level of DNA-PK.sub.cs activity that is DNA-independent (Hammarsten et al., 2000; Yaneva et al., 1997). Positions of phosphorylated proteins are indicated on the right. Bands lower than GST-Artemis represent degradation products of GST-Artemis (see also FIG. 2A).

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	159	dna adj (pk or activated adj (protein adj kinase$1 or pk))	US-PGPUB; USPAT	OR	OFF	2004/04/30 14:28
L2	49	1 same (substrate$ or peptide$)	US-PGPUB; USPAT	OR	OFF	2004/04/30 14:29
L3	32	1 same (assay$8 or detect$8 or quantit$8)	US-PGPUB; USPAT	OR	OFF	2004/04/30 14:42

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TITLE: Drug screening systems and assays

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 296014

DATE FILED: June 12, 2003

FOREIGN-APPL-PRIORITY-DATA:

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GB	0012179.8	2000GB-0012179.8	May 20, 2000

PCT-DATA:
APPL-NO: PCT/GB01/02180
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ABSTRACT:

A method of stimulating non-homologous end-joining (NHEJ) of DNA the method comprising performing NHEJ of DNA in the presence of inositol hexakisphosphate (IP.sub.6) or other stimulatory inositol phosphate. An assay of a protein kinase wherein the assay comprises inositol hexakisphosphate (IP.sub.6) or other stimulatory inositol phosphate. The invention also provides screening assays for compounds which may modulate NHEJ and which may be therapeutically useful; and screening assays for compounds which may modulate DNA-PK and related protein kinases and which may be therapeutically useful. Methods of modulating NHEJ and protein kinases are also disclosed.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

A method of stimulating non-homologous end-joining (NHEJ) of DNA the method comprising performing NHEJ of DNA in the presence of inositol hexakisphosphate (IP.sub.6) or other stimulatory inositol phosphate. An assay of a protein kinase wherein the assay comprises inositol hexakisphosphate (IP.sub.6) or other stimulatory inositol phosphate. The invention also provides screening assays for compounds which may modulate NHEJ and which may be therapeutically

useful; and screening assays for compounds which may modulate DNA-PK and related protein kinases and which may be therapeutically useful. Methods of modulating NHEJ and protein kinases are also disclosed.

Summary of Invention Paragraph - BSTX (1):
[0001] The present invention relates to assays and drug screening systems involving components of the non-homologous end joining (NHEJ) pathway, and to screening systems which make use of the protein kinase known as DNA-PK (DNA-dependent protein kinase) and related protein kinases such as ATR, ATM and FRAP. The invention also relates to inositol hexakisphosphate (IP.sub.6), inositol pentakisphosphate (IP.sub.5), inositol tetrakisphosphate (IP.sub.4), diphosphoinositol pentakisphosphate (IP.sub.7) and bis-diphosphoinositol tetrakisphosphate (IP.sub.8).

Summary of Invention Paragraph - BSTX (8):
[0008] WO 99/04266 relates to the interaction of p53 with, and its phosphorylation by, ATM and related protein kinases such as ATR and DNA-PK. The activity of the proteins is shown to increase in the presence of DNA. Assays for modulators of phosphorylation by the interaction between the proteins and p53 or other proteins having similar phosphorylation sites are provided. Methods of purifying ATM or ATR are also claimed

Summary of Invention Paragraph - BSTX (21):
[0021] The present invention makes use of these observations in order to develop further methods of performing NHEJ and assays of NHEJ; screening assays for compounds which may modulate NHEJ and which may be therapeutically useful; screening assays for compound which may modulate DNA-PK and related protein kinases and which may be therapeutically useful; compositions and kits of part which may be useful in performing the assays and methods; and methods of modulating NHEJ.

Summary of Invention Paragraph - BSTX (24):
[0024] "Non-homologous end-joining" is the ligation of DNA termini, typically intermolecular ligation. It includes the joining of DNA ends which exhibit little or no complementarity to each other (and so, typically, each end does not hybridise to the other) and, in any event, is a term well known in the art as is evidenced by its use in many of the papers and patent applications referred to herein, all of which are incorporated herein by reference. Typically, a NHEJ reaction requires a suitable DNA substrate, and suitable components for the reaction of joining the DNA ends to proceed. Suitable DNA substrates are those that, typically, are linear DNA molecules the length of which need only be large enough to accommodate the factors which participate in NHEJ. Conveniently, each DNA fragment to be joined is, independently, at least 50 bp, preferably at least 70 bp, more preferably at least 100 bp but may be bigger. In relation to the observation of a NHEJ reaction, particularly in a screening assay, one or both of the DNA molecules (or DNA ends) to be joined are detectably labelled such as with radiolabelled phosphorus or with fluorescent labels. Although it is convenient to use two separate DNA molecules to be joined in the NHEJ, two ends of the same molecule can be joined such as the ends of a linearised plasmid. NHEJ typically takes place in a eukaryotic cell, such as a vertebrate cell including mammalian cells (although it can also occur in some circumstances in prokaryotes) but, as is described in detail in Baumann & West (1998) Proc. Natl. Acad. Sci USA 95, 14066-14070, it can also occur in cell-free extracts, such as those obtained from human cells as therein described. Intermolecular ligation in this cell-free system was found to be accurate and to depend on DNA ligase IV, XRCC4 and DNA-dependent protein kinase (DNA-PK; this is a heterotrimer made up of a catalytic subunit DNA-PKcs (encoded by the XRCC7 gene) and two further subunits which are believed to be involved in DNA binding, namely Ku70 and Ku80 subunits

(which are encoded by the XRCC6 and XRCC5 genes, respectively). However, it is possible to get a low level of NHEJ with DNA ligase IV and XRCC4 in the absence of DNA-PK, but a greater extent of NHEJ is obtained when DNA ligase IV and XRCC4 are present with Ku70 and Ku80, and still further NHEJ is achieved when the catalytic subunit of DNA-PK is present. Following the inventors present work, it has now been shown that NHEJ is far better in the presence of IP.sub.6, and even better in the presence of IP.sub.7.

Summary of Invention Paragraph - BSTX (45):
[0045] The assay contains sufficient components in order to carry out NHEJ of DNA. In particular, typical assays of NHEJ are those which can be performed in vitro such as described in Baumann & West supra. NHEJ in cell-free systems is also described in Labhart (1999) Eur. J. Biochem. 265, 849-861, incorporated herein by reference. Reconstitution of NHEJ may be achieved by using recombinantly expressed protein components (such as expressed using a baculovirus system); typically, such a reconstituted system includes DNA-PK, XRCC4, DNA ligase IV, a suitable DNA substrate and a stimulatory inositol phosphate such as IP.sub.6. The assay may also be carried out in vivo using DNA substrates which, for example, are designed to observe V(D))J joining (see Smith et al (1998) J. Mol. Biol. 281, 815-825 for an example).

Summary of Invention Paragraph - BSTX (60):
[0060] A third aspect of the invention provides the use of IP.sub.6 or other stimulatory inositol phosphate for stimulating non-homologous end-joining of DNA. It is will be appreciated that before the present invention, and despite the extensive study of NHEJ, it was not realised that IP.sub.6 or other stimulatory inositol phosphate could stimulate (or may even be essential for) NHEJ. The invention also provides the use of IP.sub.6 or other stimulatory inositol phosphate in assays for compounds which modulate NHEJ by whatever means, and in methods which modulate NHEJ by whatever means. The assays may involve changes in NHEJ activity, changes in the recognition of substrates by the NHEJ components and/or changes in subcellular localisation of components of the NHEJ reaction such as DNA-PK, XRCC4 or DNA ligase IV.

Summary of Invention Paragraph - BSTX (124):
[0124] We have shown that IP.sub.6 and IP.sub.7 binds to DNA-PK. More particularly, IP.sub.6 and IP.sub.7 have been shown to bind to the Ku70/80 heterodimer which forms part of DNA-PK. Thus, it is particularly preferred that the protein kinase is a protein kinase which has a domain, preferably a C-terminal domain, with similarity to the catalytic domain of phosphoinositide 3-kinase. The protein kinase is preferably any one of the members of this family of protein kinases as discussed above. It is particularly preferred if it is DNA-PK. As noted DNA-PK is made up of three subunits. The binding assay may use any one of DNA-PK.sub.cs, Ku70 or Ku80 or, as noted above, any functional variants (eg fragments) thereof which retain the binding site for the stimulatory inositol phosphate such as IP.sub.6. Preferably, the binding assay uses the Ku70/Ku80 heterodimer.

Summary of Invention Paragraph - BSTX (149):
[0149] Compounds which, following the screening method of the ninth and tenth aspects of the invention, are ones which mimic or modulate the effect of IP.sub.6 or other stimulatory inositol phosphate in a NHEJ reaction are selected for further study. Compounds which modulate the binding of the stimulatory inositol phosphate (such as IP.sub.6) to DNA-PK (or a subunit thereof) or XRCC4 or DNA ligase IV may also be selected for further study. Conveniently, these compounds are then tested further in another screen which is designed for the selection of compounds which are suitable for treating cancer, augmenting cancer radiotherapy and/or chemotherapy regimes, improving gene therapy regimes, enhancing homologous recombination, treating retroviral

infections, or modulating the immune system. Typically, the screens are ones which involve cell-based assays which look at end-points relevant to the condition in question. The screens may also involve animal models of the relevant condition. Cell-based screens and animal models are available for at least some of cancer, augmentation of cancer radiotherapy and/or chemotherapy, gene therapy, homologous recombination, retroviral infections and immune system modulation.

Brief Description of Drawings Paragraph - DRTX (5):
[0178] FIG. 4. Stimulation of DNA-PK dependent end-joining by inositol phosphates. A. Schematic representation of IP.sub.6. B. Complementation of PC-C by IP.sub.6. End-joining assays were carried out using PC-C complemented with increasing amounts of IP.sub.6. C. Effect of inositol phosphates on DNA-PK dependent NHEJ. Inositol hexakisphosphate (IP.sub.6), inositol pentakisphosphate (IP.sub.5), inositol tetrakisphosphate (IP.sub.4), inositol trisphosphate (IP.sub.3) or inositol hexasulphate (IP.sub.6) were assayed for their ability to stimulate DNA end-joining by PC-C.

Detail Description Paragraph - DETX (52):
[0238] Binding reactions (55 .mu.l) were carried out in 25 mM HEPES pH 7.5, 50 mM KCl, 10 mM MgCl.sub.2, 1 mM DTT, 10% glycerol, 0.1% NP-40 with 5000 units of DNA-PK (Promega) or 1.8 mg/ml of protein size standards for gel filtration (BioRad) and 100 nM .sup.3H-IP.sub.6 or .sup.3H-IP.sub.3 at 4.degree. C. for 30 min. Complexes were resolved on a Superose 12 PC3.2/30 column run in 50 mM HEPES pH 8.0, 40 nM KOAc, 0.1M KCl, 10% glycerol, 1 mM DTT at 40 .mu.l/min. 50 .mu.l fractions were collected; 20 .mu.l samples of each fraction were used for .sup.3H scintilation counting (10 min/sample in 5 ml EcoscintA) and 10 .mu.l were assayed for DNA-PK kinase activity using the SignaTECT DNA-PK assay system (Promega).

Detail Description Paragraph - DETX (85):
[0264] The specificity of IP.sub.6 recognition by DNA-PK was assessed by competition trials using inositol hexasulphate (IS.sub.6). As shown in FIG. 9, tritiated IP.sub.6 (.sup.3H-IP.sub.6) is not bound by any of the molecular weight standards used to calibrate the gel filtration column (diamonds, STDs) and is detected in the far-included volume which elutes late in the column profile. Addition of DNA-PK results in an increase in the mobility of the .sup.3H-IP.sub.6 indicating complex formation. IS.sub.6, presented in 10- and 100-fold molar excess have no effect on the amount of .sup.3H-IP.sub.6 bound by DNA-PK. These data, when taken together with binding trials using .sup.3H-IP.sub.3, indicate specific binding of IP.sub.6 by DNA-PK

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TITLE: Methods for detecting dna damage and screening for cancer therapeutics

PUBLICATION-DATE: February 5, 2004

INVENTOR-INFORMATION:

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DATE FILED: January 17, 2003

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US	60208716	2000US-60208716	June 1, 2000

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DATE-FILED: May 30, 2001
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US-CL-CURRENT: 435/6

ABSTRACT:

A method for detecting DNA damage in a tissue sample involves contacting an immobilized biological sample with a labeled ligand which binds to human 53Bp1, and examining the immobilized sample for the presence of a label generated-detectable signal concentrated in foci in said sample. The presence of concentrated foci is indicative of DNA damage and the presence of diffuse signal is indicative of a normal sample. Diagnostic reagents contain a ligand that binds to human 53Bp1 associated with a detectable label. Diagnostic kits for detecting DNA damage in a biological sample contain such diagnostic reagents and signal detection components. Compositions that inhibit or antagonize the biological activity of 53Bp1 are identified by suitable assays, and are employed in methods of retarding the growth of a cancer cell.

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Detail Description Paragraph - DETX (140):

[0148] 53Bp1 has homology to the budding yeast Rad9, which is required for activation of the DNA damage checkpoint and is now proposed to participate in DNA repair through the NHEJ pathway [de la Torre-Ruiz, M., and Lowndes, N. F. 2000 FEBS Lett., 467:311-315; Weinert and Hartwell, 1988]. It is theorized that 53Bp1 has a similar role in mammalian cells. Because 53Bp1 responds early

to DNA damage, it is likely a sensor protein which is necessary for proper signaling in either the checkpoint or repair pathways. ATM is activated within minutes following exposure to DNA double strand break agents. While the peak of 53Bp1 foci occurs at 15-30 minutes after DNA damage, foci are detected within five minutes of ionizing radiation. Thus, the initial response of 53Bp1 is fast enough to be consistent with 53Bp1 being part of the ATM-Chk2 DNA damage checkpoint pathway. Alternatively, or in addition, 53Bp1 is part of the NHEJ pathway as suggested by the colocalization with Mre11/Rad50/NBS. The fact that wortmannin, an inhibitor of PI-3-like kinases, decreases the rate of 53Bp1 recruitment to sites of DNA breaks suggests that DNA-PK or ATM is involved. 53Bp1 functions in the DNA damage checkpoint or repair pathway.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040014701 A1

TITLE: Inhibiting retrotransposon and retroviral integration by targeting the atm pathway

PUBLICATION-DATE: January 22, 2004

INVENTOR-INFORMATION:

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US-CL-CURRENT: 514/44, 435/5 , 435/6

ABSTRACT:

Ataxia telangiectasia mutated (ATM)-dependent DNA damage signalling mechanisms are involved in retroviral and retrotransposon integration. Screening methods for inhibitors of retroviral and retrotranspons activity comprise inhibiting the ATM-dependent DNA damage signalling pathway, e.g. by disrupting interaction between components of the pathway. Inhibitors are useful as anti-retroviral agents, e.g. in inhibition of HIV.

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Summary of Invention Paragraph - BSTX (14):
[0014] A role for Ku and associated proteins has recently been reported in retroviral integration and the mechanistically related process of retrotransposition (Daniel et al., 1999; Downs and Jackson, 1999; WO/GB98/00099). Ku is a heterodimer of proteins of .about.70 and 80 kDa (Ku70 and Ku80 respectively), which together with the DNA-dependent protein kinase (DNA-PK) catalytic subunit, plays a pivotal role in double stranded break (DSB) repair through the DNA non-homologous end-joining (NHEJ) pathway (for review see Critchlow and Jackson, 1998). Ku has been shown to be associated with the virus-like particles (VLPs) of the yeast retrotransposable element Ty1, and potentiates retrotransposition (Downs and Jackson, 1999). Other components of the Ku-associated DNA repair pathway, such as DNA-PKcs and XRCC4 (a NHEJ protein that is thought to recruit DNA ligase IV to DNA DSBs) have also been shown to be required for efficient retroviral integration in mammalian cells

(Daniel et al., 1999). While these two studies suggest a role for the Ku-associated pathway in retroviral integration, the requirement is not absolute and residual integration events are detected in all cases (Downs and Jackson, 1999; Daniel et al., 1999).

Brief Description of Drawings Paragraph - DRTX (7):
[0099] FIG. 4 shows data from CFA and LUCIA assays which show that retroviral integration is abrogated by the DNA-PK inhibitors wortmannin and LY294002.

Brief Description of Drawings Paragraph - DRTX (9):
[0101] FIG. 6 shows the results of kinase assays which demonstrate the differential inhibition of DNA-PK and ATM kinase activity by wortmannin and LY294002.

Brief Description of Drawings Paragraph - DRTX (12):
[0104] FIG. 9 shows that the DNA-PK inhibitors wortmannin and LY294002 also inhibit the integration of HIV-1 retrovirus in LUCIA assays.

Detail Description Paragraph - DETX (11):
[0117] DNA-PK was purified from HeLa nuclear extract as described previously (Gell et al., 1999). ATM was immunoprecipitated from HeLa nuclear extract using polyclonal antisera raised to the caspase cleavage site region of ATM as described previously (Smith et al., 1999). Kinase assays were performed in 50 mM Hepes, pH 7.5, 50 mM KCl, 4 mM MnCl.sub.2, 6 mM Mg Cl.sub.2, 10% glycerol, 1 mM DTT, 1 mM NaF and 1 mM NaVO4 containing either purified DNA-PK or immunoprecipitated ATM and 1 .mu.g of the substrate GST-p53 (residues 1 to 66). Reactions were pre-incubated at 30.degree. C. for 10 minutes with varying concentrations of wortmannin or LY294002 (for final concentrations see figure). This was followed by the addition of 5 .mu.Ci of .gamma.[.sup.33P]-ATP and ATP to a final concentration of 50 .mu.M. Reactions were then incubated for a further 20 minutes at 30.degree. C. before stopping them with SDS-PAGE sample buffer. Proteins were separated by SDS-PAGE and the gels dried before using autoradiography to detect phosphorylated substrate.

Detail Description Paragraph - DETX (33):
[0139] In order to determine if ATM represents the additional target responsible for the differential activity of these two compounds in SCID cells, we looked at the effects of both wortmannin (FIG. 6A) and LY294002 (FIG. 6B) on the kinase activity of ATM. In these biochemical assays, purified DNA-PK and ATM proteins were tested in parallel for their ability to phosphorylate a bacterially expressed GST-p53 substrate in the presence of increasing concentrations of wortmannin or LY294002 (see Materials and Methods). Kinase reactions containing either purified DNA-PK or immunoprecipitated ATM and GST-p53 substrate (p53 residues 1 to 66) were performed in the presence of varying concentrations of wortmannin or LY294002 as indicated in FIG. 6.

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TITLE: Methods and kits for transferases

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INVENTOR-INFORMATION:

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APPL-NO: 10/ 199970

DATE FILED: July 19, 2002

US-CL-CURRENT: 435/15, 435/23

ABSTRACT:

A method for detecting transferase activity of a sample includes contacting the sample with a substrate and at least one of a phosphate group donor and a phosphate group acceptor. The substrate includes a reporter compound and amino acids. A peptidase is added that cleaves a non-phosphorylated substrate at a first rate and a phosphorylated substrate and a second rate. The output of the reporter compound is detected. In a preferred embodiment, the transferase activity detected is a kinase activity. In another preferred embodiment, the transferase activity detected is a phosphatase activity. Also provided is a method of screening for alterations in a transferase reaction. Kits and peptide substrate are also provided for carrying out at least one of the methods of the invention.

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Detail Description Paragraph - DETX (52):

[0089] Activators can be added to the kinase reaction where desired, e.g., where the kinase under investigation requires an activator. It also may be desirable to add an activator to achieve optimal kinase activity. Activators useful in the kinase reaction include, but are not limited to, calcium, phospholipids and other lipids, and phorbol 12-myristate 13-acetate (PMA) or similar activators for Calcium-phospholipid-dependent protein kinase (PKC), calcium and calmodulin for calmodulin-dependent protein kinase (CaM K), cAMP for cAMP-dependent protein kinase (PKA) holoenzyme, cGMP for cGMP-dependent protein kinase (PKG), DNA for DNA-PK. Activators can be added at nanomolar or higher concentrations and at micromolar or lower concentrations depending on the kinase under investigation. A termination reagent can optionally be added to the system in which the kinase reaction is occurring where an end point is desired, e.g., for measuring and quantitating the activity of protein kinase. The termination reagent usually is a metal chelating reagent added at a concentration that is sufficient to sequester the metal away from the kinase. In addition, any other reagent that terminates the phosphorylation catalyzed by the kinase can be used to terminate the phosphorylation reaction. For example, EDTA, EGTA, and 1,10-phenanthroline are good chelators for magnesium, calcium, and zinc, respectively. Other ion chelating agents may be used. Additionally,

kinases can be heat inactivated.

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DOCUMENT-IDENTIFIER: US 20040005586 A1

TITLE: Novel Artemis/DNA-dependent protein kinase complex and methods of use thereof

PUBLICATION-DATE: January 8, 2004

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/6, 435/199 , 435/91.2

ABSTRACT:

In the present invention, it is disclosed that Artemis forms a complex with the 469 kDa DNA-dependent protein kinase (DNA-PK.sub.cs) in vitro and in vivo in the absence of DNA. The purified Artemis protein alone possesses single-strand specific 5' to 3' exonuclease activity. Upon complex formation, DNA-PK.sub.cs phosphorylates Artemis, and Artemis acquires endonucleolytic activity with respect to single-stranded nucleotides, including 5' and 3' overhangs, as well as hairpins. Further, the Artemis:DNA-PKcs complex can open hairpins generated by the RAG complex from a 12/23-substrate pair. Thus, DNA-PK.sub.cs regulates Artemis by both phosphorylation and complex formation to permit enzymatic activities that are critical for the hairpin opening step of V(D)J recombination and for all of the 5' and 3' overhang processing in nonhomologous DNA end joining.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 60/355,452, filed Feb. 6, 2002, and to U.S. Provisional Application Serial No. 60/360,659, filed Feb. 28, 2002.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (18):

[0051] FIG. 10 is an autoradiogram of a DNA-PK kinase assay in which a 35 bp DNA was used as the DNA-PKcs cofactor.

Detail Description Paragraph - DETX (98):

[0138] Nuclease assays without RAGs were carried out in a total volume of 10 .mu.L with a buffer composition of 25 mM Tris, pH 8.0, 10-50 mM NaCl or KCl, 10 mM MgCl.sub.2, 1 mM DTT, and 50 ng/.mu.L of BSA unless otherwise specified. To the buffer mixture, Artemis was added to 2.75 pmol, and DNA-PK.sub.cs and Ku were added to 1.25 pmol each. 0.25 mM of ATP (or ADP, ATP-y-S, AMP-PNP) and 0.5 PM of 35 bp DNA were included where DNA-PK.sub.cs was used. Reactions were incubated at 37.degree. C. for 30 minutes. In reactions including DNA-PK.sub.cs inhibitors, reaction mixtures without the substrate were incubated on ice for 15 minutes before the addition of the substrate and the subsequent incubation at 37.degree. C. In FIG. 7, pre-phosphorylation of Artemis-myc-His immunobeads was carried out under DNA-PK kinase assay conditions. After washing the treated immunobeads with buffer F for three times and the nuclease assay buffer for two times, the beads were used for the nuclease reactions. In the hairpin opening of RAG-generated hairpins (FIG. 8), the reactions contained 25 mM K-HEPES, pH 7.4, 50 mM KCl, 10 mM MgCl.sub.2, 1 mM DTT, 0.25 pmol of labeled 12-RSS double-stranded oligonucleotides (KY28/KY29) and an equal amount of unlabeled 23-RSS double-stranded oligonucleotides (KY36/KY37), 1 pmol of RAGs (assuming that the RAG complex consists of two RAG-1 and two RAG-2 subunits), 2 pmol of HMG1, 2.75 pmol of Artemis-myc-His, and 1.25 pmol of DNA-PK.sub.cs (with ATP and 35 bp DNA as described above). For the sequential reactions, substrates were incubated with RAG complex alone first at 37.degree. C. for 60 minutes, extracted with or without phenol/chloroform, then Artemis-myc-His and DNA-PK.sub.cs were added, followed by another 30-minute incubation at 37.degree. C. Reactions with the RAG complex, Artemis, and DNA-PK.sub.cs added simultaneously were incubated for 90 minutes at the same temperature. After incubation, reactions were stopped by adding an equal volume of formamide gel loading buffer and heating at 100.degree. C. for 5 minutes. DNA was resolved on 12% denaturing polyacrylamide gels. The gels were then dried and exposed to a PhosphorImager screen. Data was analyzed by ImageQuant software (v5.0).

Detail Description Paragraph - DETX (100):

DNA-PK Kinase Assay

Detail Description Paragraph - DETX (115):

[0147] A DNA-PK kinase assay was performed to determine whether Artemis is a phosphorylation substrate of DNA-PK.sub.cs. The results are shown in FIG. 10. DNA-PK.sub.cs was incubated alone (i.e., with no protein substrate; lanes 1 and 2), or with DNA ligase IV/XRCC4 (positive control, lanes 3 and 4) or GST-Artemis (lanes 5 and 6). The low amount of XRCC4 and Artemis phosphorylation in the absence of 35 bp dsDNA is thought to be due to a low level of DNA-PK.sub.cs activity that is DNA-independent (Hammarsten et al., 2000; Yaneva et al., 1997). Positions of phosphorylated proteins are indicated on the right. Bands lower than GST-Artemis represent degradation products of GST-Artemis (see also FIG. 2A).

Detail Description Paragraph - DETX (116):

[0148] The results of the DNA-PK kinase assay demonstrated that Artemis is indeed a prominent phosphorylation target of DNA-PK.sub.cs, as illustrated by the DNA dependent phosphorylation (lanes 5 and 6). Therefore, DNA-PK.sub.cs not only forms a physical complex with Artemis, but it is also able to efficiently phosphorylate Artemis upon complex formation. The results further show that this activity is dependent on DNA ends. These results imply that the Artemis: DNA-PK.sub.cs nuclease complex would be ideally responsive to pathologic dsDNA breaks.